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Nitrogen partitioning among photosynthetic components and its consequence in sun and shade plants

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Summary

1. *Chenopodium album* (a sun species) and *Alocasia macrorrhiza* (a shade species) plants were grown under various photon flux densities (PFDs) to investigate whether nitrogen partitioning among photosynthetic components was optimized under any light conditions. The amounts of several photosynthetic components of the leaves were determined to examine nitrogen partitioning.

2. For the same PFD, nitrogen partitioning among photosynthetic components was similar in both species, except for leaves of *C. album* grown at 5% PFD, which showed a markedly smaller fraction of nitrogen in photosystem I.

3. Optimal nitrogen partitioning among photosynthetic components was estimated for various PFDs using a simulation model of leaf photosynthesis. At any PFD, the actual nitrogen partitioning was very similar to the estimated optimal partitioning. However, partitioning in *C. album* grown at 5% PFD deviated from the optimum.

4. For both species grown under any light conditions, estimated daily photosynthesis of actual leaves was very close to that of leaves with optimal partitioning. It is concluded that both species achieve nitrogen partitioning leading to nearly maximum carbon gain under any light condition.

Key-words: Acclimation, leaf nitrogen, optimization, photosynthetic apparatus

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Introduction

The range of light environments within which plants can survive, grow and reproduce differs among species. Many physiological and morphological traits of a given species can potentially determine its range of favourable light environments. Because photosynthesis is the sole metabolism supplying carbon in higher plants, it is expected that adaptation and/or acclimation of photosynthesis will play an important role in delimiting their habitat.

Nitrogen is one of the resources that limit plant growth. Because more than half the leaf nitrogen is allocated to photosynthetic proteins (Makino & Osmond 1991a; Evans 1989a), the efficient use of nitrogen in photosynthetic systems in accordance with the environment may be important in enhancing the fitness of species (Field & Mooney 1986).

Some theoretical studies have shown that changes in the organization of the photosynthetic apparatus with changes in the growth environment contribute to an increase in photosynthetic production by enhancing the nitrogen-use efficiency of photosynthesis. Using a simple model, Evans (1989b) suggested that there is a trade-off relationship between maximum rate and initial slope of the light response curve of photosynthesis in relation to nitrogen partitioning within the leaf. Hikosaka & Terashima (1995) further developed a model to summarize the roles of all the photosynthetic components. They predicted that, at high light, increase in the allocation of nitrogen to Calvin cycle enzymes, coupling factor and electron carriers, all of which are important in determining the light-saturated rate of photosynthesis, will result in an improvement of daily photosynthesis. Under low light conditions, on the other hand, increase in allocation to chl protein complexes, which are involved in the use of low PFDs, will have an advantage. The predicted changes in the organization of the photosynthetic apparatus were consistent with those observed in actual leaves (Hikosaka & Terashima 1995; Hikosaka 1996). Evans (1989b, 1993) suggested that actual nitrogen partitioning in some cultivar species was similar to the optimal partitioning.

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However, although some studies have already compared nitrogen utilization within leaves of sun and shade species grown under various PFDs (Seemann *et al.* 1987; Sims & Pearcy 1989), it remains unclear whether the acclimation of the photosynthetic apparatus in sun and shade species to contrasting PFDs is always optimal. It is still possible that limitations in the ability to acclimate restricts the range of habitats that these species can occupy. In the present study, the acclimation of the photosynthetic apparatus of two species, *Chenopodium album* (a sun species) and *Alocasia macrorrhiza* (a shade species) was investigated. The plants studied were grown under various light conditions, and the question of whether nitrogen partitioning among photosynthetic components is a factor responsible for the habitat range of sun and shade species was investigated.

Materials and methods

GROWTH CONDITIONS

Chenopodium album L. is a broad-leaved summer annual and generally colonizes disturbed habitats. *Alocasia macrorrhiza* (L). G. Don is an understorey perennial. Both species have been frequently used in physiological studies (e.g. Sage & Pearcy 1987; Chow *et al.* 1988; Seemann *et al.* 1987; Sims & Pearcy 1989). Seeds of *C. album* were collected in Tokyo (see Nagashima, Terashima & Katoh 1995). *A. macrorrhiza* plants used were collected at Okinawa Island, Japan. Plants were grown in a natural-light greenhouse. The range of air temperature in the greenhouse in daytime was 20–30 °C. Plants were grown in pots (1·5 l volume) filled with vermiculite, which were placed in plastic trays (four pots per tray). Each tray contained 6 l nutrient solution (12 mM NO_3^-, see Hikosaka, Terashima & Katoh 1994 for detailed composition). The nutrient solution was continuously aerated and was renewed every week.

Five irradiances (5, 10, 27, 49 and 100% of full sunlight), which were adjusted with shade cloth, were established. *A. macrorrhiza* is known to be tolerant to photoinhibition when plants are grown under strong light (Seemann *et al.* 1987). *A. macrorrhiza* plants were grown under these five irradiances. *C. album* plants, grown under full sunlight for a month, were transferred to each of these five conditions and grown further for another month. Newly expanded leaves, produced after the transfer, were used for the experiments. PFD in the greenhouse was monitored every 5 min with photodiodes (Hamamatsu Photonics, Hamamatsu, Japan) which were calibrated against a quantum sensor (Li-Cor, Lincoln, USA).

MEASUREMENT OF PHOTOSYNTHESIS

The rate of CO_2 exchange of the attached leaf was determined with an open gas-exchange system (for detail, see Hikosaka 1996). Plants were kept in a dark room for at least 10 h before the measurement of photosynthesis. The dark respiration rate was measured first at ambient CO_2 (partial pressure 35 Pa) and irradiance was increased in steps until photosynthesis was light saturated. Leaf temperature was adjusted to 25 ± 1 °C (range). Vapour pressure deficit was less than 1 kPa.

DETERMINATION OF CHLOROPHYLL AND NITROGEN CONTENTS

After measurement of photosynthesis, leaves were washed with distilled water and four discs of 1-cm diameter were punched out from one leaf. The residues were stored at –80 °C until determination of ribulose 1,5-bisphosphate carboxylase (RuBPCase). Chlorophyll and nitrogen contents were determined by the methods of Hikosaka (1996).

DETERMINATION OF RUBPCASE CONTENT

RuBPCase was determined according to Makino, Mae & Ohira (1986) with some modifications. The frozen leaf was homogenized in 100 mM sodium-phosphate buffer (pH 7·5) containing 0·4 M sorbitol, 10 mM ascorbate, 2 mM $MgCl_2$, 10 mM NaCl, 1 mM phenylmethylsulphonyl fluoride and 5 mM iodine acetate. The homogenate was filtered through 20-μm mesh, RuBPCase concentration in the filtrate was determined according to Hikosaka (1995). Calibration curves were made with the RuBPCase purified from *Spinacia oleracea* L., determined by the method of Lowry *et al.* (1951).

DETERMINATION OF CONTENTS OF THYLAKOID COMPONENTS

The rest of the above filtrate was centrifuged at 2000 *g* for 8 min. The supernatant was used for the measurement of soluble protein content (see below). The pellet was suspended in 100 mM sodium phosphate buffer (pH 6·5) containing 0·4 M sorbitol, 2 mM $MgCl_2$ and 10 mM NaCl. The suspension was stored at –80 °C and used as thylakoid membranes. Thylakoid nitrogen was determined with a NC analyser (Shimadzu, Kyoto, Japan).

The photo-oxidizable concentration of P700 (the reaction centre of photosystem I), the amplitude of the absorbance change $\Delta A_{540\text{-}550}$ (C550; one C550 per photosystem II reaction centre) and concentration of cytochrome *f* (cyt *f*) were determined as described in Hikosaka (1996).

DETERMINATION OF SOLUBLE PROTEIN CONTENT

The above-mentioned supernatant was further centrifuged at 30 000 *g* for 30 min. Trichloroacetic acid (TCA) was added to the supernatant (final concentration, 10% TCA) and kept for 30 min at room tempera-

ture. The mixture was centrifuged at 3000 *g* for 10 min. The pellet was washed with ethanol and was solubilized in 0·25M NaOH. The protein content of this NaOH solution was determined by the micro-biuret method (Itzhaki & Gill 1964).

Calculations

NITROGEN COST OF PHOTOSYNTHETIC COMPONENTS

Nitrogen costs of the photosynthetic components are calculated by the method of Hikosaka & Terashima (1995). Photosynthetic components are categorized into six groups: (A) LHCII; (B) PSII core; (C) PSI; (D) electron carriers and coupling factor; (E) Calvin-cycle enzymes other than RuBPCase; (F) RuBPCase. Groups (D) and (E), which were categorized as one group (Group II) by Hikosaka & Terashima (1995) are treated separately in order to consider nitrogen partitioning between stroma and thylakoid membrane. However, cyt *f* is used as the representative for the amount of nitrogen in both of these groups (see Hikosaka & Terashima 1995).

Contents of RuBPCase, cyt *f*, PSII core (C550) and PSI (P700) are determined experimentally. The amount of LHCII per unit leaf area is derived from the equation:

$$\text{LHCII} = (1000\ \text{chl} - 184\ \text{PSI} - 60\ \text{PSII})/13, \quad \text{eqn 1}$$

where components denote their amounts per unit area (μmol m^{-2}, except for chl, mmol m^{-2}), 1000 is a factor for adjustment, and 184, 60 and 13 denote numbers of chl molecules bound to PSI, PSII core and LHCII, respectively (see Hikosaka & Terashima 1995).

The amount of nitrogen in each group of components is found by multiplying the amount of each group by its unit nitrogen cost n_x (Table 1), multiplied by the amount of the representative component. The value of n_x is calculated assuming that 16% of the mass of protein is nitrogen. Associated chl molecules (four nitrogen atoms per chl molecule) are also taken into account (Table 1).

Photosynthetic nitrogen, N_p, is the sum of nitrogen in all the components:

$$N_p = n_A\,\text{LHCII} + n_B\,\text{PSII} + n_C\,\text{PSI} + (n_D + n_E)\,\text{cyt}f + n_F\,\text{RuBPCase}, \quad \text{eqn 2}$$

where n_x is the nitrogen cost of the group x in mol N per μmol of the representative component except for n_F, mol N g^{-1} protein (for values, see Table 1); units for cyt *f* and RuBPCase are μmol m^{-2} and g m^{-2}, respectively.

SIMULATION

The light-response curve of photosynthetic rate is expressed as a non-rectangular hyperbola (Thornley 1976):

$$P = \frac{[\phi I + P_{max} - \{(\phi I + P_{max})^2 - 4\phi I\theta P_{max}\}^{1/2}]}{2\theta} - R, \quad \text{eqn 3}$$

Table 1. Nitrogen cost of photosynthetic components and the number of chlorophyll molecules associated with the various proteins. A, LHCII; B, PSII core; C, PSI (core+LHCI); D, electron carriers and coupling factor; E, Calvin-cycle enzymes other than RuBPCase; F, RuBPCase (see text and Hikosaka & Terashima (1995) for more detailed explanations)

Groups	Mass of protein (kDa)	N cost ($10^6\ n_x$) (mol)	Binding chl (mol)
A	25	338	13
B	417*	5000*	60
C	465*	6040*	184
D	475†	5420†	0
E	359†	4100†	0
F	550	6290‡	0

*Expressed as mol^{-1} reaction centre.
†Expressed as mol^{-1} cyt *f*.
‡Expressed as mol^{-1} RuBPCase. However, in this study, n_F = 0·0114 mol N g^{-1} protein is used because RuBPCase is expressed as g m^{-2}.

where P is the net photosynthetic rate, I is the incident PFD, P_{max} is the light-saturated rate of gross photosynthesis, ϕ is the initial slope of the curve, R is the rate of dark respiration, and θ is the convexity of the curve.

Dependencies of P_{max} or ϕ on each component are expressed as following equations, assuming that P_{max} is *co-limited* by all the related components.

$$P_{max} = a_r\,\text{RuBPCase} / (b_r + \text{RuBPCase}); \quad \text{eqn 4}$$

$$P_{max} = a_f\,\text{cyt}f; \quad \text{eqn 5}$$

$$P_{max} = a_p\,\text{PSII}; \quad \text{eqn 6}$$

$$\phi = a_c\,\text{chl} / (b_c + \text{chl}), \quad \text{eqn 7}$$

where a_x and b_x are constants (see Table 2 for values and units). These equations mean that the amounts of groups A, B and C determine ϕ (because they contain chl molecules) and that the amounts of groups C, D, E and F determine P_{max}.

The amount of PSI is assumed to be proportional to that of chl:

$$\text{PSI} = a_s\,\text{chl}. \quad \text{eqn 8}$$

P_{max} is a function of RuBPCase (equation 4); after appropriate arrangements, ϕ can be expressed as a function of RuBPCase and N_p (see Appendix 2). Thus, when R and θ are known, photosynthetic rate P is expressed as a function of three parameters, I, RuBPCase and N_p. The value of R used was the mean measured respiration rate for leaves grown under the respective light conditions; θ was fixed at 0·9. Leaf temperature and ambient CO_2 were assumed to be 25 °C and 35 Pa, respectively. Measured PFD was used for I to calculate P, and daily carbon exchange rate (CER) was calculated as the sum of P.

In the simulation to obtain optimal partitioning at a given PFD, N_p is estimated from the experimental

Table 2. Constants used in calculations

Parameter	Unit	Value	
		A. macrorrhiza	*C. album*
θ	—	0·9	0·9
a_r	μmol m^{-2} s^{-1}	33·9	127·8
b_r	g m^{-2}	4·2	13·2
a_f	mol mol^{-1} s^{-1}	17·6	26·4
a_p	mol mol^{-1} s^{-1}	12·0	16·1
a_s	mol $mmol^{-1}$	2·80	2·48
		2·37*	1·76*
a_c	mol mol^{-1}	0·065	0·065
b_c	mmol m^{-2}	0·076	0·076

*These values are used in the calculation for CER at 5% PFD.

data for leaves grown at that PFD, using equation 2. Because amounts of other groups can be expressed as functions of the amounts of RuBPCase and N_p, only the amount of RuBPCase is varied to obtain the optimal partitioning. The ratio of RuBPCase nitrogen to N_p (*RN*) is used as an index of nitrogen partitioning.

Results

PHOTOSYNTHETIC PROPERTIES

Figure 1 shows several photosynthetic properties expressed on a leaf-area basis. Except for *C. album* leaves grown at 5% and 10% PFD and *A. macrorrhiza* leaves grown at 100% PFD (Fig. 1a), leaf nitrogen content decreased with decreasing irradiance. P_{max}, the sum of light-saturated rate of photosynthesis at 35 Pa of ambient CO_2 and respiration rate in dark-adapted leaves, decreased with decreasing irradiance (Fig. 1b). There was little change in leaf chl content with decreasing PFD except at 5% PFD, where it was lower in both species (Fig. 1c). Contents of RuBPCase in both species decreased strongly with decrease in PFD. However, in *A. macrorrhiza* leaves grown at 100% PFD the RuBPCase contents were similar to those of leaves grown at 49% PFD (Fig. 1d).

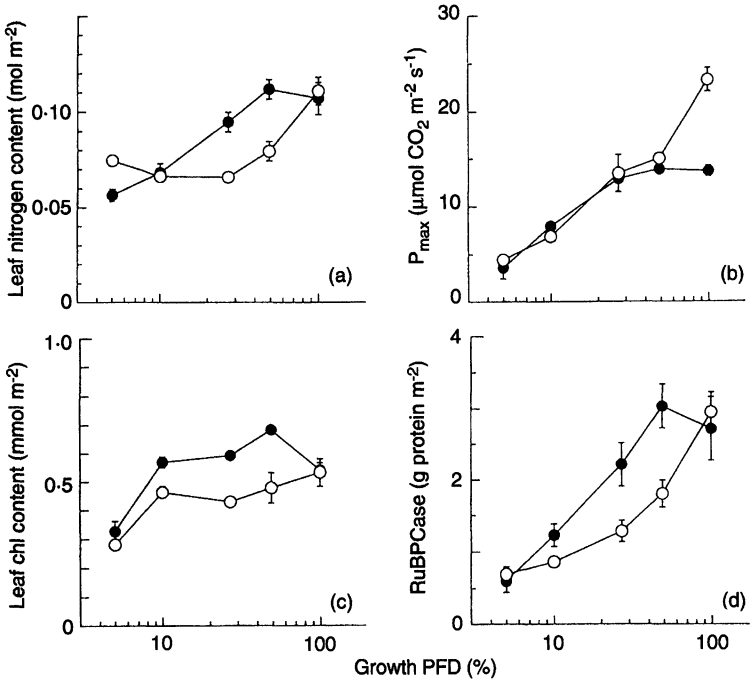

Fig. 1. Photosynthetic properties of *A. macrorrhiza* (closed circles) and *C. album* (open circles) plotted against growth PFD: nitrogen content (a), P_{max} (b), chl content (c) and RuBPCase (d) per unit leaf area. Means ± SD of three leaves are shown.

Figure 2 shows the chl *a/b* ratios and contents of thylakoid components expressed on a chl basis. Chlorophyll *a/b* ratios decreased with decrease in irradiance (Fig. 2a). The changes were more marked in *C. album*. PSI per chl (one P700 per PSI) was almost independent of growth irradiance except for the value of *C. album* leaves grown at 5% PFD, which was significantly lower than others (Fig. 2b). PSII per chl (one C550 per PSII) decreased with a decrease in growth PFD down to 10% PFD. However, for both species, the values at 5% PFD were greater than those at 10% PFD (Fig. 2c). Cyt *f* per chl also decreased with decrease in growth PFD except for *C. album* leaves grown at 5% PFD (Fig. 2d).

NITROGEN PARTITIONING IN LEAVES

Figure 3 compares nitrogen allocation in both species. Contents of soluble protein and RuBPCase are plotted against the leaf nitrogen content. The RuBPCase content was about half the soluble protein content in both species. There were no apparent differences in dependence of the contents of soluble protein or RuBPCase on the leaf nitrogen content between *C. album* and *A. macrorrhiza*. However, soluble protein and RuBPCase contents in *C. album* leaves grown at 5% PFD were relatively low (see below). The dependency of the cyt *f* content on the nitrogen content was also similar for both species (data not shown).

Figure 4 compares nitrogen utilization in the leaves. Except for *C. album* leaves grown at 5% and 10% PFD, photosynthetic nitrogen, N_p (A+B+...+F), was more than half the leaf nitrogen. RuBPCase (F) was the component with the most influence on the difference in N_p; the total amounts of chl-protein complexes (A+B+C) were relatively constant irrespective of growth PFD. The nature of 'other nitrogen' (H) is unclear. In any sample, the fraction of nitrate was less than 4% of leaf nitrogen (data not shown). 'Other nitrogen' in *C. album* increased with decrease in growth PFD.

In Figure 4 (c, d), relative nitrogen partitioning within N_p is shown. The fraction of nitrogen in RuBPCase was largest in leaves grown at high PFD levels. The fractions of nitrogen in LHCII (A) and PSI (C) increased with decrease in growth PFD. However, changes in fractions of nitrogen in other three groups, other C_3 enzymes (E), electron carriers and CF (D), and PSII core (B) were relatively small irrespective of growth PFD. *C. album* grown at 5% PFD was exceptional because nitrogen partitioned into PSI was

markedly low. Except for this case, nitrogen partitioning was similar in leaves of both species grown at the same PFD.

In order to assess the validity of some assumptions in the present model, measured chl *a/b* ratios and thylakoid nitrogen contents were compared to calculated values (Fig. 5). The chl *a/b* ratio is calculated assuming that *a/b* ratios of PSI, PSII core and LHCII are 8, 11 and 1·1, respectively (Hikosaka & Terashima 1995). Although the chl *a/b* ratios used are not accurate (see Hikosaka & Terashima 1995), estimated values of chl *a/b* ratios of leaves were almost identical to measured values (Fig. 5a). The thylakoid nitrogen was estimated as A+B+C+D. The relationship between estimated and measured thylakoid nitrogen also showed a strong correlation but deviated from a 1 : 1 relationship (Fig. 5b).

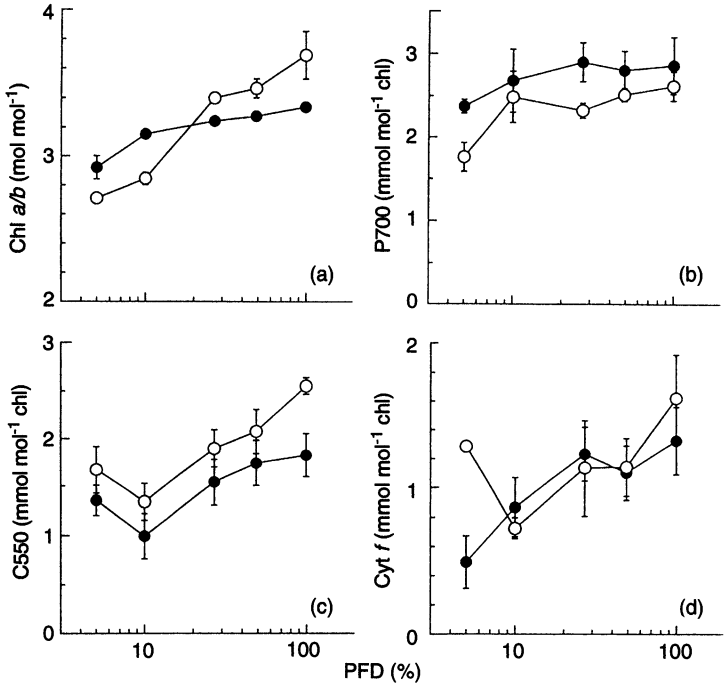

Fig. 2. Chlorophyll *a/b* ratio (a) and the amounts of photosynthetic components P_{700} (b), C_{550} (c) and cyt *f* (d) in leaves of *A. macrorrhiza* and *C. album.* Symbols are as in Fig. 1.

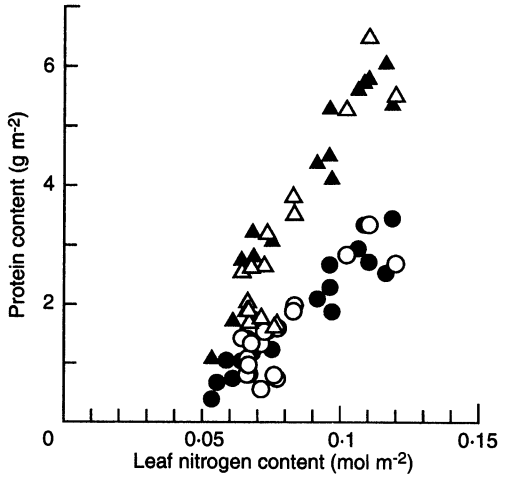

Fig. 3. Relationship between contents of protein and nitrogen. Triangles and circles denote soluble protein and RuBPCase, respectively. Closed and open symbols denote *A. macrorrhiza* and *C. album,* respectively.

SIMULATION

For the simulation, the dependencies of P_{max} on photosynthetic components were examined (see equations 4, 5 and 6). Parameter values are shown in Table 2. Significant correlations were observed between contents of RuBPCase, cyt *f* and PSII core and P_{max} in both species (Fig. 6).

In the simulation, the values of N_p shown in Fig. 4 were used. N_p for a given species under a given irradiance was fixed to the value determined by actual measurement and only partitioning was varied. Changes in irradiance in the greenhouse were monitored every 5 min from 8 to 2 days before the sampling. There were 4 sunny days (maximum PFD 1000–1200 μmol m^{-2} s^{-1}; total PFD 13·3–17·0 mol m^{-2} day^{-1}) and 2 cloudy days (maximum 280 and 500 μmol m^{-2} s^{-1}; total 2·9 and 6·9 mol m^{-2} day^{-1}). Photosynthetic rates were calculated every 5 min and integrated to obtain daily carbon exchange rate (CER). Optimal partitionings that give maximum daily CER for each of 6 days and that give maximum CER during 6 days were numerically calculated.

Figure 7 compares the actual and optimal nitrogen partitionings. The ratio of nitrogen in RuBPCase to N_p (*RN*) is used as the index of nitrogen partitioning. Bars indicate the range of the maximum and minimum values of *RN* giving maximum daily CER (RN_{day}) for each of 6 days. The *RN* values giving maximum CER during 6 days (RN_{avg}) are also calculated. RN_{avg} increased with increase in PFD. Except for *C. album* leaves grown at 5% PFD, actual partitionings were included in the range of RN_{day} and very close to RN_{avg}.

Effects of nitrogen partitioning on average daily CER for 6 days are shown in Figure 8. Daily CER varied greatly depending on nitrogen partitioning. Average daily CER values during 6 days were also calculated for each of the PFD with actual N_p value for the PFD but with different *RN* values that were observed in the leaves grown under different PFDs. The ratio of average CER for each *RN* to that for RN_{avg} is plotted as a function of PFD (Fig. 9). It is clear that the nitrogen partitioning among photosynthetic components can cause a significant reduction in carbon gain when the partitioning is very different from the optimal or the observed partitioning for the PFD under consideration. For example, in both species, CER at 5% PFD calculated for the *RN* of the leaves grown at 100% PFD was about 20% lower than that for the RN_{avg} or that for the observed *RN* at 5% PFD. However, for all the leaves that are acclimated to their growth PFD, the carbon gain was very close to that with the optimal partitioning.

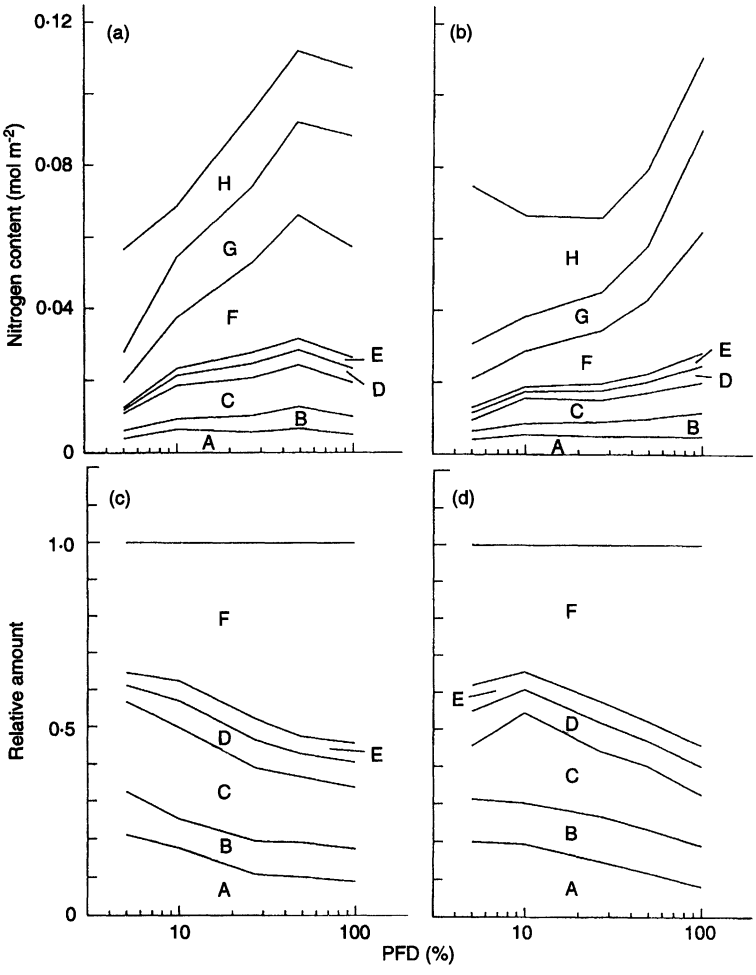

Fig. 4. (a, b) Nitrogen partitioning within leaves of *A. macrorrhiza* (a) and *C. album* (b). A, LHCII; B, PSII core; C, PSI; D, electron carriers and coupling factor; E, Calvin-cycle enzymes other than RuBPCase; F, RuBPCase; G, other soluble proteins; H, other nitrogen. The amount of LHCII is calculated from equation 1. Nitrogen contents in photosynthetic components (A, B, C, D, E and F) are calculated according to equation 2. E+F+G equals soluble protein. H is determined as leaf nitrogen – (A+B+C+D+ soluble protein). (b, c) Relative nitrogen partitioning among photosynthetic components: (c) *A. macrorrhiza*; (d) *C. album*. Amount of photosynthetic nitrogen (N_p) is normalized.

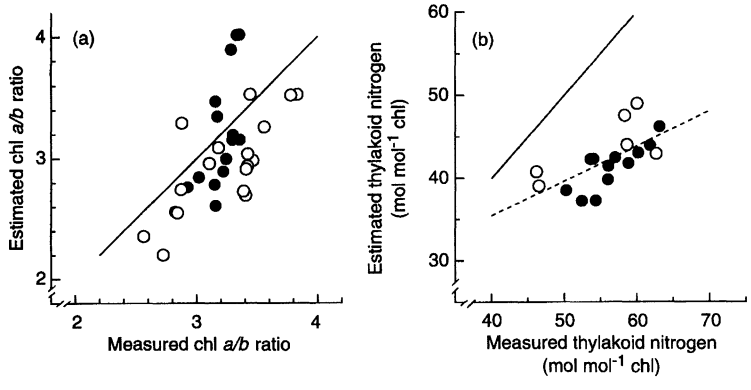

Fig. 5. Relationships between estimated and measured quantities. (a) The chl *a/b* ratio of leaves. The solid line denotes 1 : 1 correspondence. (b) Thylakoid nitrogen per unit chl. The solid line denotes 1 : 1 correspondence. The broken line is a regression (Y = 0·42 X + 18·7, r = 0·65). Symbols are as in Fig. 1.

Discussion

NITROGEN PARTITIONING IN LEAVES OF SUN AND SHADE SPECIES

So far, two comparative studies of the photosynthesis–nitrogen relationships of sun and shade species have been published. Seemann *et al.* (1987) raised plants of *A. macrorrhiza* and *Phaseolus vulgaris* (a sun species) under various light conditions and found that, at a given nitrogen content, the content of RuBPCase on a leaf area basis in *P. vulgaris* leaves was almost twice that of *A. macrorrhiza* leaves. Conversely, the chl content was higher in *A. macrorrhiza* leaves. These results imply that *P. vulgaris* achieves high carbon gain under high PFD conditions, whereas *A. macrorrhiza* has an advantage at low PFD. Therefore, these differences have been considered to be important factors in segregation of habitats between sun and shade species.

However, the data obtained by Sims & Pearcy (1989) were not consistent with the results of Seemann *et al.* (1987) They compared gas-exchange characteristics of *A. macrorrhiza* and *Colocasia esculenta* (a sun species) grown under various light conditions. The dependence of the maximum rate of carboxylation, which may be proportional to the amount of RuBPCase, on the leaf nitrogen content was very similar for both species. The results obtained in the present study also clearly demonstrate that the dependence of RuBPCase contents on the leaf nitrogen content was almost identical between *A. macrorrhiza* and *C. album* (Fig. 3). The dependence observed in the present study almost corresponded to that of *P. vulgaris* in Seemann *et al.* (1987). It is unclear why such contradictory results were obtained in spite of the fact that the same species, *A. macrorrhiza*, was used. However, it is noteworthy that only the amount of soluble RuBPCase was determined in the experiment by Seemann *et al.* (1987). It is known that part of RuBPCase is often tightly bound to thylakoid membranes (Makino & Osmond 1991b) and the fraction varies depending on growth treatments such as sink removal and shading (Crafts-Brandner, Salvucci & Egli 1991). It is therefore possible that Seemann *et al.* underestimated the amount of RuBPCase in *A. macrorrhiza* leaves.

It is also possible that the affinity of CBB for RuBPCase differs depending on species. However, homology in the amino acid sequence of the large subunit of RuBPCase between Chenopodiaceae and Araceae is *ca.* 93% (N. Murakami, personal communication). Therefore, the affinity of CBB to RuBPCase would not differ much between these species. The fact that the relationship between soluble protein and nitrogen was similar in both species (Fig. 3) also suggests that the allocation of nitrogen within leaves is similar in both species.

ACCLIMATION OF THE PHOTOSYNTHETIC APPARATUS

Changes in the photosynthetic apparatus of the two species, depending on PFD, are consistent with previous observations (see Anderson 1986; Terashima & Takenaka 1986; Anderson, Chow & Goodchild 1988; Terashima & Evans 1988). However, some of the results were inconsistent. For example, relatively high ratios of PSII/chl, cyt *f*/chl and RuBPCase/chl were observed in *C. album* leaves grown at 5% PFD (Fig. 2). From Fig. 4(d), it seems likely that these high ratios are due to the small amount of chl bound to PSI (see also Fig. 2b). Small decreases in PSI/chl may occur with decreasing PFD (Anderson 1986). However, it is unclear how balanced excitations of PSI and PSII are possible in such plants. Although the number of chl molecules per PSI is assumed to be fixed in the present study (see Hikosaka & Terashima 1995), it is possible that the LHCI/PSI ratio increases with decreasing PFD (Anderson 1986).

The calculations presented in this paper would overestimate the amount of LHCII if LHCI/PSI and therefore the amount of chl bound to PSI increased with decreasing PFD. However, because the nitrogen cost and the number of chl molecules bound to LHCI are presumably similar to those of LHCII (Jansson 1994), estimated nitrogen partitioning will be valid if it is assumed that 'LHCII' in Fig. 4 contains the 'excess LHCI'.

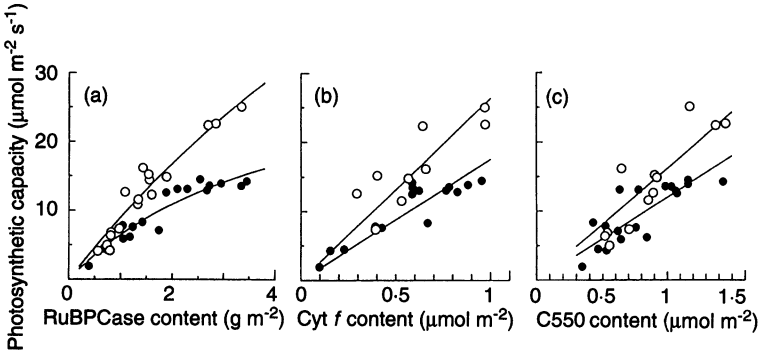

Fig. 6. Relationships between P_{max} and RuBPCase (a), cyt *f* (b) and C550 (c). Lines are regressions: (a) $Y = 33{\cdot}89\,X/(4{\cdot}22 + X)$, $r = 0{\cdot}95$ (*A. macrorrhiza*) and $Y = 127{\cdot}81\,X/(13{\cdot}22 + X)$, $r = 0{\cdot}96$ (*C. album*); (b) $Y = 17{\cdot}6X$, $r = 0{\cdot}79$ (*A. macrorrhiza*) and $Y = 26{\cdot}4X$, $r = 0{\cdot}87$ (*C. album*); (c) $Y = 12{\cdot}0X$, $r = 0{\cdot}80$ (*A. macrorrhiza*) and $Y = 16{\cdot}1X$, $r = 0{\cdot}88$ (*C. album*).

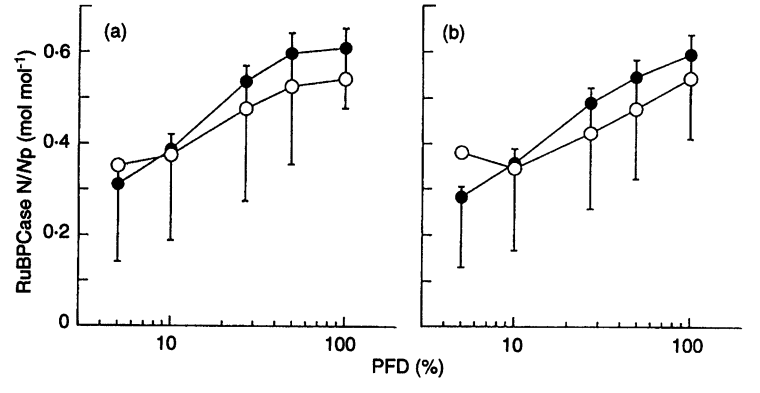

Fig. 7. Optimal and actual partitioning of photosynthetic nitrogen. The ratio of nitrogen in RuBPCase to photosynthetic nitrogen (*RN*) is used as an index. (a) *A. macrorrhiza*; (b) *C. album*. Bar indicates the range of maximum and minimum of six *RN* values that give maximum daily CO_2 exchange rate (RN_{day}) under the PFD conditions on each of 6 days (8–2 days before sampling). Closed circles denote the *RN* that gives maximum CO_2 exchange rate during 6 days (RN_{avg}). Open circles denote actual partitioning.

VALIDITY OF ASSUMPTIONS OF THE MODEL

Because it is difficult to determine the content of all the photosynthetic components, four representative components were selected and some assumptions were used to determine nitrogen partitioning among photosynthetic components (Hikosaka & Terashima 1995). Strong correlations between estimated and measured values of chl *a/b* ratio and thylakoid nitrogen suggest that the assumptions are valid (Fig. 5).

Measured thylakoid nitrogen was, however, greater than the estimate (Fig. 5b). Two possible reasons can be suggested. One is that isolated thylakoid membranes contain nitrogenous compounds other than photosynthetic components. This could be the case; calculated values include only photosynthetic proteins. The other reason is invalid estimation of the amount of other components, such as coupling factor. Amounts of coupling factor and Calvin-cycle enzymes other than RuBPCase are estimated assuming that they are proportional to the amount of cyt *f*. Although many studies support this assumption (Evans 1987; Terashima & Evans 1988; Chow & Anderson 1987; Makino, Nakano & Mae 1994), it is unclear whether the stoichiometry is common among species. The values of cyt *f*/chl observed in the present study were small relative to those previously reported (e.g. 2·7 mmol mol^{-1} in sun leaves of spinach, Terashima & Evans 1988; 2–3 mmol mol^{-1} in sun leaves of *Ipomoea tricolor*, Hikosaka 1996). Therefore, it is possible that amounts of Calvin-cycle enzymes other than RuBPCase, electron carriers other than the cyt *b/f* complex, and coupling factor are underestimated. We also estimated optimal nitrogen partitioning for the case in which their amounts are doubled (18 mol N μmol^{-1} cyt *f* was assumed). RN_{avg} decreased and became closer to the actual *RN* (data not shown). However, since nitrogen in these components is still small relative to N_p, these changes were very small and the conclusion of the present study is not markedly affected.

OPTIMIZATION OF THE ORGANIZATION OF THE PHOTOSYNTHETIC APPARATUS

At any PFD, carbon gain in leaves with nitrogen partitioning among photosynthetic components for contrasting PFD can be 10-20% lower than that of the optimal partitioning (Fig. 9); this result clearly suggests that nitrogen partitioning is of ecological importance. Except for *C. album* leaves grown at 5% PFD,

nitrogen partitioning among photosynthetic components at a given PFD was similar in both species (Fig. 4) and the actual partitioning was very similar to the optimal partitioning (Fig. 7). These results strongly suggest that these species have ability to adjust the organization of the photosynthetic apparatus to a large range of light environments. Although the light conditions of the habitats of these species are different, the difference in the ability to adjust partitioning under a given light condition is very small and may not be a factor delimiting their habitat.

The actual partitioning in *C. album* leaves grown at 5% PFD deviated from the optimal partitioning (Fig. 7b). This result implies that sun species lack an ability to regulate the organization of photosynthetic components optimally at very low irradiance. However, loss of daily CER calculated for the leaves with suboptimal acclimation is very small (Fig. 9) because dependency of daily CER on nitrogen partitioning near its maximum is small (Fig. 8). Thus, although inability to regulate photosynthetic organization at low growth PFD may be of physiological interest, the deviation from the optimum may not be responsible for delimiting the habitat of *C. album*.

The P_{max} of *C. album* leaves grown at 100% PFD was higher than in *A. macrorrhiza*, although the amounts of RuBPCase were comparable (Fig. 1). Sims & Pearcy (1989) also reported that the P_{max} of *Colocasia esculenta* leaves was higher than that of *A. macrorrhiza* leaves. They attributed this difference to lower stomatal conductances in *A. macrorrhiza* leaves. In the present study, stomatal conductances were not determined but it is possible that the conductance was lower in *A. macrorrhiza* leaves. The lower photosynthetic capacity in *A. macrorrhiza* can be the factor delimiting the habitat of this species because photosynthetic gain in *A. macrorrhiza* leaves at 100% PFD is much lower than in *C. album* leaves, although they have similar nitrogen content (Figs 1a and 8).

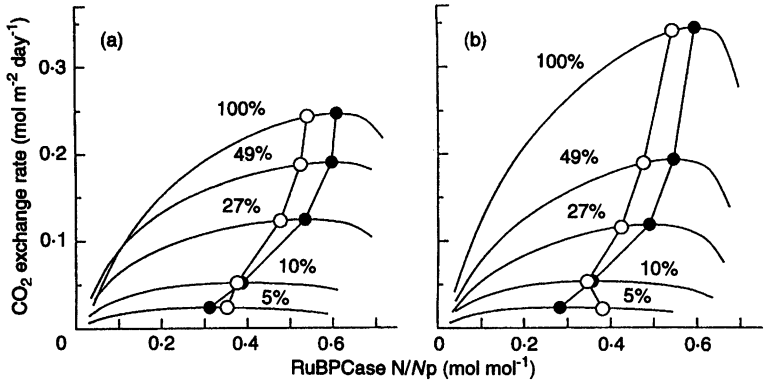

Fig. 8. Effects of nitrogen partitioning on daily CO_2 exchange rate. (a) *A. macrorrhiza*; (b) *C. album*. Daily CO_2 exchange rate is the average of daily CO_2 exchange rates calculated from 8 to 2 days before the sampling for each of the PFD conditions. Open and closed circles denote actual and optimal (RN_{avg}) partitionings.

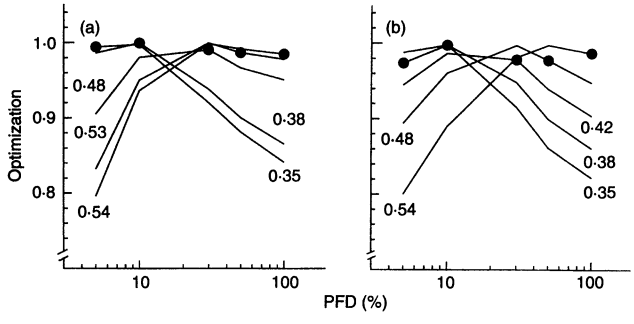

Fig. 9. Daily CO_2 exchange rate calculated with the actual N_p for a given PFD but with actual partitioning realized at all five PFDs relative to that for the optimal partitioning for the given PFD. (a) *A. macrorrhiza*; (b) *C. album*. Each continuous line denotes the changes in the ratio with the fixed RuBPCase N/N_p depending on the PFD used for calculations. In *A. macrorrhiza* 0·35, 0·38, 0·48, 0·53 and 0·54 are *RN* values estimated for leaves grown at 5, 10, 27, 49 and 100% PFD, respectively. In *C. album*, 0·38, 0·35, 0·42, 0·48 and 0·54 are values estimated for leaves grown at 5, 10, 27, 49 and 100% PFD, respectively. Each circle indicates that the carbon gain of the actual leaves is calculated for their growth PFD. Values are calculated from the data shown in Fig. 8.

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Appendix 1. List of abbreviations

a_x, b_x, regression coefficients (see Table 2)
C550, amplitude of the absorbance change $\Delta A_{540-550}$ (one C550 per photosystem II reaction centre)
CBB, Coomassie Brilliant Blue R-250
CER, (daily) carbon exchange rate on a leaf-area basis (mol CO_2 m^{-2} day^{-1})
CF, coupling factor
chl, chlorophyll (amount of chl is expressed as mmol m^{-2})
chl_x, number of chl molecules associated with chl-protein complex X
cyt *f*, cytochrome *f* (amount of cyt *f* is expressed as μmol m^{-2})
I, incident photon flux density (μmol quanta m^{-2} s^{-1})
LHC, light-harvesting complex (amount of LHCII is expressed as μmol m^{-2})
N_p, nitrogen in all the photosynthetic components
n_x, nitrogen cost; see Table 1
P, rate of net photosynthesis on a leaf area basis (μmol CO_2 m^{-2} s^{-1})
P700. reaction centre of photosystem I
PFD, photon flux density (μmol m^{-2} s^{-1})
P_{max}, light-saturated rate of gross photosynthesis on a leaf area basis (μmol CO_2 m^{-2} s^{-1})
PSI, core complex and light harvesting complex of photosystem I (amount of PSI is expressed as μmol m^{-2})
PSII core, core complex of photosystem II (amount of PSII is expressed as μmol m^{-2})
R, rate of dark respiration (μmol CO_2 m^{-2} s^{-1})
RN, ratio of nitrogen in RuBPCase to N_p
RN_{avg}, ratio of nitrogen in RuBPCase to N_p which gives maximum CER during 6 days
RN_{day}, ratio of nitrogen in RuBPCase to N_p which gives maximum daily CER
RuBPCase, ribulose 1,5-bisphosphate carboxylase (amount of RuBPCase is expressed as g m^{-2})
TCA, trichloroacetic acid
ϕ, initial slope of the light-response curve of photosynthesis (mol CO_2 mol^{-1} incident quanta)
θ, convexity of the light-response curve of photosynthesis (no dimension)

Appendix 2

Substituting equations 1, 4, 5, 6, 7 and 8 into equation 2 after appropriate arrangements, N_p can be expressed as a function of ϕ and RuBPCase;

$$N_p = \frac{b_c \phi}{a_c - \phi}\left(\frac{1000\, n_A + 13 a_s n_C - 184\, a_s n_A}{13}\right) + \frac{a_r\,\text{RuBPCase}}{b_r + \text{RuBPCase}}\left(\frac{13\, n_B - 60\, n_A}{13\, a_p} + \frac{n_D + n_E}{a_f}\right) + n_F\,\text{RuBPCase} \qquad \text{eqn A1}$$

ϕ can be expressed as follows:

$$\phi = \frac{a_c\,(V(\text{RuBPCase}) - N_p)}{V(\text{RuBPCase}) - N_p - b_c W} \qquad \text{eqn A2}$$

where $W = (1000\, n_A + 13\, a_s\, n_C - 184\, a_s\, n_A)/13$, and V(RuBPCase) is expressed as:

$$V = \frac{a_r\,\text{RuBPCase}}{b_r + \text{RuBPCase}}\left(\frac{13\, n_B - 60\, n_A}{13\, a_p} + \frac{n_D + n_F}{a_f}\right) + n_F\,\text{RuBPCase}.$$

Other groups can also be expressed as functions of RuBPCase or RuBPCase and N_p (not shown).